
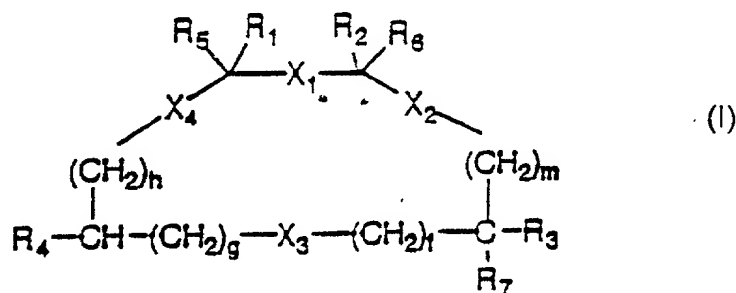


U.S. APPLICATION NO. (if known, see 37 CFR 1.51)		INTERNATIONAL APPLICATION NO. PCT/EP98/00599		ATTORNEY'S DOCKET NUMBER 515-4167		
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$970.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$840.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$760.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$670.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$96.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY		
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$		
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE			
Total claims	14 - 20 =	0	X \$18.00	\$	0	
Independent claims	2 - 3 =	0	X \$78.00	\$	0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			0	+ \$260.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$	840.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$	0	
SUBTOTAL =				\$	840.00	
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TOTAL FEES ENCLOSED =				\$	840.00	
				Amount to be:	\$	
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a. <input checked="" type="checkbox"/> A check in the amount of \$ <u>840.00</u> to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>08-1540</u> . A duplicate copy of this sheet is enclosed.						
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.						
SEND ALL CORRESPONDENCE TO JAMES V. COSTIGAN, ESQ. HEDMAN, GIBSON & COSTIGAN, P.C. 1185 AVENUE OF THE AMERICAS, SUITE 2003 NEW YORK, NY 10036-2646 212-302-8989						
				 SIGNATURE	JAMES V. COSTIGAN	
				NAME	25,669	
				REGISTRATION NUMBER		

MONOCYCLIC COMPOUNDS WITH FOUR BIFUNCTIONAL RESIDUES HAVING NK-2 ANTAGONIST ACTION

Scope of the invention

The present invention refers to new compounds having the general formula (I):



in which:

X_1, X_2, X_3, X_4 , which may be the same or different from one another, represent a group chosen from among -CONR-, -NRCO-, -OCO-, -COO-, -CH₂NR-, -NR-CH₂-, CH₂-CH₂-, where R is H or a C₁₋₃ alkyl or benzyl;

f, g, h, m, which may be the same or different from one another, represent a number chosen from among 0, 1 or 2;

R_1 and R_2 , which may be the same or different from one another, represent a $-(CH_2)_r$ -Ar group, where $r = 0, 1, 2$ and where Ar is an aromatic group chosen from among: benzene, naphthalene, thiophene, benzothiophene, pyridine, quinoline, indole, furan, benzofuran, thiazole, benzothiazole, imidazole, and benzo-imidazole, the said Ar group being possibly substituted with a maximum of 2 residues chosen from among C₁₋₃ alkyl or halo-alkyl, C₁₋₃ alkoxy, C₂₋₄ amino-alkoxy, halogen, OH, NH₂, NR₁₃R₁₄ where R_{13} and R_{14} , which may be the same or different from one another, represent hydrogen or C₁₋₃ alkyl;

R_3 represents a group chosen from among:

- hydrogen
- linear or branched alkyl having the formula C_nH_{2n+1}, with $n = 1-5$, cyclo-alkyl or alkylcyclo-alkyl groups having the formula C_nH_{2n-1} with $n = 5-9$
- $(CH_2)_r$ -Ar₁, where $r = 0, 1, 2$ and where Ar₁ is an aromatic group chosen from among: benzene, naphthalene, thiophene, benzothiophene, pyridine, quinoline, indole, furan, benzofuran, thiazole, benzothiazole, imidazole, and benzo-imidazole, the said Ar₁ group being possibly substituted with a maximum of 2

residues chosen from among C_{1-3} alkyl or halo-alkyl, C_{1-3} alkoxy or amino-alkoxy, halogen, OH, NH_2 , $NR_{13}R_{14}$, where R_{13} and R_{14} , which may be the same or different from one another, represent hydrogen or C_{1-3} alkyl;

R_4 represents a group chosen from among:

- 5 - hydrogen or C_{1-6} alkyl
- L-Q, where L is a chemical bond or a linear or branched C_{1-6} alkyl residue and Q is a group chosen from among:
 - i) H, OH, OR_9 , NH_2 , NR_9R_{10} , guanidine, sulphate, phosphonate, phosphate, where R_9 and R_{10} , which may be the same or different from one another, represent a hydrogen, C_{1-3} alkyl group, C_{1-3} hydroxyalkyl, C_{1-3} dihydroxyalkyl, C_{1-3} alkyl- $CONHR_{12}$, C_{1-3} alkyltetrazole, C_{1-3} alkyl-COOH or wherein R_9R_{10} joined together form with the N-atom a saturated 4-6 membered heterocycle possibly containing a further heteroatom chosen in the group consisting of N, O, S and wherein R_{12} is a mono-, di-, tri-glycosidic group possibly protected with one or more C_{1-3} -acyl groups or substituted with amino-groups or C_{1-3} acylamino-groups;
 - 15
 - 20
 - 25
 - 30
 - ii) COOH, tetrazole, SO_2NH_2 , $SO_2NHCOOR_8$, $CONHR_8$, $NHCOR_8$, where R_8 represents a linear or cyclic C_{1-6} alkyl chain containing one or more polar groups chosen from among the group: OH, NH_2 , $NR_{15}R_{16}$, COOH, $CONHR_{12}$, PO_3H , SO_3H , OR_{11} and where R_{15} and R_{16} , which may be the same or different from one another, represent a hydrogen or C_{1-3} alkyl group, and where R_{11} is a C_{1-3} alkyl or C_{2-4} amino-alkyl chain, R_{12} is a mono-, di-, tri-glycosidic group possibly protected with one or more C_{1-3} acyl groups or substituted with amino-groups or C_{1-3} acylamino-groups or $R_{15}R_{16}$ joined together form with the N-atom a saturated 4-6 membered heterocycle possibly substituted with C_{1-3} alkyl-groups or with saturated 4-6 membered heterocycle-groups containing at least an N-atom;
 - iii) $COOR_{17}$, $CONHR_{12}$, OR_{12} where R_{12} is a mono-, di- or tri-glycoside group possibly protected with one or more C_{1-3} acyl groups or substituted with amine or C_{1-3} acylamine groups and R_{17} is a group R_{12} as above defined or a group C_{1-3} alkyl, C_{1-3} alkylphenyl, wherein the phenyl-group can be substituted with a group OH, NO_2 , NH_2 , CN, CH_3 , Cl, Br;

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R₅, R₆, R₇, which may be the same or different from one another, represent a hydrogen or C₁₋₃ alkyl group; with the proviso that when R₁ and R₂ are benzyl or 4-hydroxybenzyl then R₃ and R₄ are not isopropyl.

Also included in the present invention are the pharmaceutically acceptable salts, the processes for their preparation, and the pharmaceutical compositions containing them.

In view of the presence of chiral centres in the compounds of formula (I), also the individual enantiomers and their mixtures, both in the racemic form and in the non-racemic form, form part of the present invention.

10 State of the art

The NK-2 receptor of tachykinins is widely expressed in the peripheral nervous system of mammals. One of the various effects produced by the selective stimulation of the NK-2 receptor is the contraction of smooth muscle. Hence antagonists of the NK-2 receptor may be considered agents capable of controlling excessive contraction of smooth muscle in any pathological condition in which the release of tachykinins concurs in the genesis of the corresponding disorder.

In particular, the bronchospastic and inflammatory component of asthma, coughing, pulmonary irritation, intestinal spasms, spasms of the biliary tract, local spasms of the bladder and of the ureter during cystitis, kidney infections and colics may be considered conditions in which the administration of NK-2 antagonists may be effective (E.M. Kudlacz *et al.*, Eur. J. Pharmacol., 1993, 241, 17-25).

In addition, a number of NK-2 antagonists capable of surmounting the haemato-encephalic barrier have shown anxiolytic properties (D.M. Walsh *et al.*, Psychopharmacology, 1995, 121, 186-191).

Cyclic compounds, and in particular cyclic hexapeptides (A.T. McKnight *et al.*, Br. J. Pharmacol., 1991, 104, 355) and bicyclic hexapeptides (V. Pavone *et al.*, WO 93/212227) or cyclic hexapeptideptides (L. Quartara *et al.*, J. Med. Chem., 1994, 37, 3630; S.L. Harbeson *et al.*, Peptides, Chemistry and Biology. Proceedings of the Twelfth American Peptide Symposium, 1992, 124) are known in the literature for their antagonistic activity towards the NK-2 receptor

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of tachykinins. In EP-A-333174 linear di-and tri-peptide compounds comprising the -D-Trp-Phe-moiety are described; such compounds possess tachykinin antagonism.

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AMENDED SHEET

It has now surprisingly been found that products of lower molecular weight, monocyclic ones, containing only four bifunctional residues linked together via peptide or pseudopeptide bond, present high pharmacological activity associated to a considerable selectivity for the human NK-2 receptor, and thus are proposed as valid alternatives.

Detailed description of the invention

The present invention therefore sets itself the aim of making available new monocyclic compounds containing four bifunctional residues linked together via peptide or pseudopeptide bonds having antagonistic action on the NK-2 receptor, with the general formula (I), as defined previously.

Also forming part of the present invention are the pharmaceutically acceptable salts, the processes for their preparation, and the pharmaceutical compositions containing them.

In view of the presence of chiral centres in the compounds of formula (I), also the individual enantiomers and their mixtures, both in the racemic form and in the non-racemic form, form part of the present invention.

According to the invention preferred compounds of general formula (I) are those in which:

f, g, h, m, which may be the same or different from one another, may be 0 or 1;

R₁ and R₂, which may be the same or different from one another, represent the side chain of a natural amino acid chosen from among tryptophan, phenyl alanine, tyrosine, histidine or the side chain of a non-natural amino acid chosen in the group:

tryptophan and phenyl alanine, either mono- or di-substituted with residues chosen from among C₁₋₃ alkyl or halo-alkyl, C₁₋₃ alkoxy or amino-alkoxy, halogen, OH, NH₂, NR₁₃R₁₄, where R₁₃ and R₁₄, which may be the same or different from one another, represent a hydrogen or C₁₋₃ alkyl group;

R₃ represents a group chosen from among:

- linear or branched alkyl having the formula C_nH_{2n+1}, with n = 1-5 (chosen in the group consisting of methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl) cycloalkyl or alkylcycloalkyl of formula C_nH_{2n-1} with n = 5-9 (chosen in the group consisting of cyclopentyl, cyclohexyl, methylcyclohexyl)

- $(\text{CH}_2)_r\text{-Ar}_1$, where $r = 1$ or 2 and where Ar_1 is an aromatic group chosen from among: α -naphthyl, β -naphthyl, phenyl, indole, the said Ar_1 group being possibly substituted with a maximum of 2 residues chosen from among C_{1-3} alkyl, CF_3 , C_{1-3} alkoxy, Cl, F, OH, NH_2 ;

5 R_4 represents an L-Q group where:

L is a chemical bond or CH_2 , and

Q is a group chosen from among:

- OH, NH_2 , NR_9R_{10} , OR_{11} , and where R_9 and R_{10} , which may be the same or different from one another, represent a hydrogen or C_{1-3} alkyl group, C_{1-3} hydroxy alkyl, C_{1-3} dihydroxyalkyl, C_{1-3} alkyl- CONHR_{12} wherein R_{12} is a monoglycosidic group derived from D or L pentoses or hexoses (chosen in the group consisting of ribose, arabinose, glucose, galactose, fructose, glucosamine, galactosamine and their N-acetylated derivatives), C_{1-3} alkyltetrazole, C_{1-3} alkyl-COOH or wherein R_9R_{10} are joined together to form with the N atom a morpholine or a piperidine ring and where R_{11} is a C_{1-3} alkyl chain, or a C_{2-4} amino-alkyl chain;

- NHCOR_8 wherein R_8 is a cyclohexane containing from 2 to 4 OH groups, a C_{1-6} alkylchain containing a polar group (chosen in the group consisting of NH_2 , COOH, CONHR_{12} (wherein R_{12} is as hereabove defined) or [1,4']bipiperidine)

- COOH, COOR_{17} or CONHR_{12} , wherein R_{12} is as hereabove defined and R_{17} is as R_{12} or a group 4-nitrobenzyl and R_{12} is a monoglycosidic group derived from D or L pentoses or hexoses (chosen in the group consisting of ribose, arabinose, glucose, galactose, fructose, glucosamine, galactosamine and their N-acetylated derivatives).

- R_5 , R_6 , R_7 are H.

25 Likewise preferred are isomers that present an R configuration on the carbon atom that carries the R_3 and R_7 substituents.

Pharmaceutically acceptable salts of compounds of formula (I) include the salts with inorganic acids (such as, hydrochloric acid, hydrobromic acid, hydrogen iodide, sulphuric acid, nitric acid, phosphoric acid) and organic acids (such as, acetic, propionic, succinic, malonic, citric, tartaric, metasulphonic, *para*-toluenesulphonic acids), as well as salts of pharmaceutically acceptable bases, both inorganic (such as, hydroxides of sodium, potassium, calcium,

magnesium, zinc and aluminium) and organic bases (such as, amines like methyl amine, diethyl amine, triethyl amine, ethyl amine, tromethamine or piperidine).

According to the invention, the compounds of formula (I) containing peptide or
5 pseudopeptide bonds may be obtained by means of classical condensation methods using techniques known in the literature. The general method chosen by us for preparing the peptide compounds ($X_1-X_4 = -CONR-$, $-NRCO-$) involves the synthesis in solution of the linear peptide chain using amino acids, dicarboxyl or diamine derivatives suitably protected and, after selective
10 deprotection of the C- and N-terminals, cyclization in polar organic solvents in diluted solution. As method of activation of the carboxyl groups, that using PyBOP and DIEA in DMF or HBT and EDC in DMF are generally preferred.

To provide an example, the attached diagram presents the general synthesis of compounds of formula (I) in which $X_1 = X_2 = X_3 = X_4 = -CONH-$.

15 The dicarboxyl precursors 7 containing the R_4 group, and the diamine precursors 4 containing the R_3 and R_7 groups were prepared using methods described in the literature.

In particular, the synthesis of the succinic derivatives, with $R_4 = \text{alkyl}$ or $(CH_2)_n\text{-Ar}$, is described by R. Conrow *et al.*, J. Org. Chem., 1986, 51, 938 and by S.G.
20 Cohen *et al.*, J. Am. Chem. Soc., 1968, 90, 3495, whilst in the case of $R_4 = \text{H}$, amine group, hydroxyl group or carboxyl group, the following were respectively used: succinic anhydride, aspartic acid, malic acid or carboxysuccinic acid appropriately protected.

The synthesis of the ethylene diamine derivatives containing the R_3 , R_7 groups
25 was performed starting from the corresponding N-protected amino acids by reduction of the carboxyl to alcohol with BH_3 .THF (C.F. Stanfield *et al.*, J. Org. Chem., 1981, 46, 4797, 4799; I.R. Ollmann *et al.*, Bioorg. Med. Chem., 1995, 3, 969), conversion to azide via the corresponding mesylate and subsequent reduction to amino group (P.G. Mattingly, Synthesis, 1990, 366; P.M. O'Brien *et al.*, J. Med. Chem., 1994, 37, 1810).
30

The compounds containing reduced peptide bonds ($X_1-X_4 = -CH_2-NR-$, $-NR-CH_2-$) were synthesized in solution according to known methods, such as

reductive amination of the aldehyde of an amino acid with the amine function of a protected amino acid or peptide, in the presence of NaCNBH_4 as reducer in DMF/AcOH (K.A. Jacobson *et al.*, J. Med. Chem., 1983, 26, 492; R.F. Borch *et al.*, J. Am. Chem. Soc., 1971, 93, 2897; J.P. Salvi *et al.*, Tetr. Lett., 1994, 35, 1181). The aldehydes were obtained by reduction with LiAlH_4 of the
5 corresponding protected amino acids, N,O-dimethylhydroxy-amates according to the method described by J.A. Feherentz *et al.*, Synthesis, 1983, 676 and Int. J. Peptide Res., 1985, 26, 236.

The compounds of formula 10 wherein R_4 is NH_2 or COOH can be derivatized
10 into compounds of formula 1 wherein R_4 is NR_9R_{10} , guanidine, tetrazole, NHCOR_8 , CONHR_8 , COOR_{17} , CONHR_{12} , wherein R_9 , R_{10} , R_8 , R_{12} and R_{17} are as above defined, according to known methods.

The compounds of formula (I) as specified above have proved to be powerful antagonists of the NK-2 receptor of tachykinins, and hence can be administered
15 as agents capable of controlling any central or peripheral manifestation due to excessive activation of tachykinergic neurons, and in particular excessive contraction of smooth muscle in any pathological condition in which release of tachykinins concurs in the genesis of the corresponding disorders.

In particular, the bronchospastic and inflammatory component of asthma, of coughing, of pulmonary irritation, intestinal spasms, spasms of the biliary tract,
20 and local spasms of the bladder and ureter in the course of cystitis and kidney infections and colics may be considered conditions in which the administration of the compounds of formula (I), as NK-2 antagonists, may prove effective.

The use as anxiolytic agents should also be considered for those compounds
25 that are provided with the appropriate chemico-physical characteristics for penetration into the CNS.

The compounds of formula (I) that are the subject of the present invention are suited for administration for therapeutic purposes to higher animals and man through the parenteral, oral, inhalational and sublingual routes, achieving
30 pharmacological effects according to the properties described above. For administration through parenteral (intravenous, intramuscular, and intradermal) routes, sterile or lyophilized preparations are used. As far as the nasal,

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inhalational and sublingual instillation routes are concerned, aqueous solutions, aerosol preparations, powders or capsules are used according to the particular case.

The doses of active principle in the aforesaid compositions may range between
5 0.02 and 10 mg/kg of body weight.

The invention will now be illustrated in the examples that follow, which, however, have no limiting effect.

Example 1

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂C₆H₅)-CH₂-NH-]}

10 (compound of formula (I) where: X₁ = X₂ = X₃ = X₄ = -CO-NH-; R₁ = -CH₂-(indol-3-yl); R₂ = R₃ = -CH₂-C₆H₅; R₄ = R₅ = R₆ = R₇ = H; m = h = 0, f = g = 1; the carbon atoms C-R₁ and C-R₂ have an S configuration, whereas C-R₃ has an R configuration)

a) Synthesis of BOC-Trp-Phe-OH dipeptide

15 Di-*tert*-butyl carbonate (3.4 g) was added to a solution of H-Trp-Phe-OH (5 g) in dioxane (30 ml), H₂O (15 ml) and NaOH 1M (15.6 ml), cooled to 0-5°C under stirring. The reaction mixture was kept stirred for 2 hours, and then concentrated and extracted with pentane (2 x 20 ml). The aqueous phase was cooled with ice, with the addition of AcOEt (50 ml), KHSO₄ to obtain pH 2-3,
20 separated and extracted with AcOEt (2 x 50 ml). The re-united organic phases were washed with brine (50 ml), vacuum dried and evaporated at 30°C to obtain 6 g of the desired compound as a white semi-solid residue.

TLC: r.f. 0.55 (chloroform / cyclohexane / AcOH / H₂O = 45 / 45 / 5 / 5), 0.52 .
(CHCl₃ / MeOH = 9/1)

25 b) Synthesis of (R)-1-benzyl-2-benzyloxycarbonylamino-ethyl amine

The synthesis was carried out following the method described by P.G. Mattingly, Synthesis, 1990, 366, starting from BOC-D-phenylalaninol

c) Synthesis of BOC-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH-Z] (5)

(R)-1-benzyl-2-benzyloxycarbonylamino ethyl amine (750 mg), PyBOP (1.37 g),
30 and DIEA (0.9 ml) were added to a solution of BOC-Trp-Phe-OH (1.19 g, 2.63 mmol.) in anhydrous DMG (10 ml) under nitrogen.

5 The crude compound was purified by washing in suspension with AcOEt under heat and with MeOH at room temperature to obtain 1.15 g of the desired product 5 as a white solid. MS(TS) : $[MH^+]$ = 718

10 TFA (6 ml) was added, under stirring and at room temperature, to a suspension of the compound 5 (1.1 g) in CHCl_3 (30 ml), and a clear solution was seen to form immediately. The reaction mixture was kept stirred for 1.5 hours, and the disappearance of the precursor was monitored by means of HPLC analysis. After evaporation of the solvent, the residue was diluted with AcOEt (100 ml), washed with NaHCO_3 5% (2 x 30 ml) and brine (30 ml).

The solid residue was purified by means of flash-chromatography ($\text{CHCl}_3/\text{MeOH} = 95/5$) to obtain 821 mg of the desired compound 6 as a white solid.

20 e) Synthesis of HO-Suc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH-Z] (compound 8 where: PG₂ = OH; PG₁ = Z)

After evaporation of the solvent, the residue was suspended in H₂O and kept stirred for 5 minutes. The solid was filtered and washed in suspension twice using MeOH to obtain 242 mg of the desired compound 8 as a white solid.

30 f) Synthesis of HO-Suc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH₂] (9)

The compound 8 (225 mg) was suspended in MeOH (10 ml) and hydrogenated in the presence of Pd/C 10% (50 mg) at atmospheric pressure and room

temperature. HPLC analysis after 4 hours showed that the precursor had disappeared completely.

The catalyst was filtered and washed with MeOH. After evaporation of the solvent, 158 mg of the desired compound 9 were obtained as a white solid.

5 m.p. = 142-4°C; TLC: r.f. 0.70 (*n*-butanol / AcOH / H₂O = 6 / 2 / 2)

g) Synthesis of cyclo{Suc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH-]}(10)

PyBOP (145 mg) and DIEA (0.09 ml) were added to a solution of compound 9 (148 mg) in anhydrous DMF (5 ml) stirred under nitrogen.

10 The reaction mixture was kept stirred for 5 hours and, after evaporation of the solvent, the residue was suspended in AcOEt, kept stirred for 10 minutes, and filtered, to obtain 100 mg of a solid product.

Part of the product (50 mg) was purified by HPLC to obtain 18 mg of the desired compound 10 as a white solid.

15 MS (TS) : [MH⁺] = 566; 1H-NMR (DMSO): d 2.15-2.35 (m, 2H), 2.55-2.85 (m, 8H), 2.96-3.04 (m, 2H), 3.90-4.02 (m, 1H), 4.03-4.15 (m, 1H), 4.25-4.42 (m, 1H), 6.71 (d, 1H), 6.90-7.42 (m, 16H), 8.09 (m, 1H), 8.50 (d, 1H), 10.82 (s, 1H).

Following the procedure described in Example 1, the compounds specified below were obtained:

Example 2

20 Cyclo{-Suc-Trp-Phe-[(S)-NH-CH(CH₂C₆H₅)-CH₂-NH-]}

(compound of formula I in which the substituents are defined as in Example 1, but all the C-R₁, C-R₂ and C-R₃ atoms have an S configuration) 1H-NMR (DMSO): d 1.95-2.32 (m, 2H), 2.34-2.90 (m, 6H), 2.92-3.18 (m, 2H), 3.60-3.82 (m, 1H), 4.00-4.40 (m, 4H), 6.90-7.36 (m, 14H), 7.39-7.54 (m, 2H), 7.64 (d, 1H), 25 7.88 (t, 1H), 8.27 (d, 1H), 10.78 (s, 1H).

Example 3

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂C₆H₁₁)-CH₂-NH-]}

(compound of formula I in which X₁ = X₂ = X₃ = X₄ = CO-NH-; R₁ = -CH₂-(indol-3-yl); R₂ = -CH₂-C₆H₅; R₃ = -CH₂-C₆H₁₁; R₄ = R₅ = R₇ = H; m = h = 0, f = g = 1; 30 the carbon atoms C-R₁ and C-R₂ have an S configuration, whereas C-R₃ has an R configuration) 1H-NMR (DMSO): d 0.65-0.95 (m 2H), 1.00-1.38 (m, 6H), 1.45-1.75 (m, 5H), 2.05-2.30 (m, 2H), 2.40-2.85 (m, 6H), 3.20-3.60 (m, 1H), 3.61-

3.78 (m, 1H), 3.80-3.96 (m, 1H), 3.98-4.10 (m, 1H), 4.38-4.55 (m, 1H), 8.47 (d, 1H), 6.90-7.45 (m, 11H), 8.02 (m, 1H), 8.47 (d, 1H), 10.78 (d, 1H).

Example 4

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂C₆H₄(4-OCH₃))-CH₂-NH-]}

- 5 (compound of formula I in which X₁ = X₂ = X₃ = X₄ = CO-NH-; R₁ = -CH₂-(indol-3-yl); R₂ = -CH₂-C₆H₅; R₃ = -CH₂-C₆H₄(4-OCH₃); R₄ = R₅ = R₆ = R₇ = H; m = h = 0, f = g = 1; the carbon atoms C-R₁ and C-R₂ have an S configuration, whereas C-R₃ has an R configuration) 1H-NMR (DMSO): d 2.13-2.37 (m, 2H), 2.50-2.85 (m, 8H), 3.25-3.50 (m, 1H), 3.58-3.80 (m, 4H), 3.85-4.00 (m, 1H), 4.02-4.18 (m, 10
1H), 4.28-4.45 (m, 1H), 6.65-7.47 (m, 16H), 8.02-8.16 (m, 1H), 8.48 (d, 1H), 10.80 (s, 1H).

Example 5

Cyclo{-Suc-Trp(5F)-Phe-[(R)-NH-CH(CH₂C₆H₅)-CH₂-NH-]}

- (compound of formula I, in which R₁ = -CH₂-(5-fluoroindol-3-yl), and the other
15 substituents are as defined in Example 1) MS(ES⁺): [MH⁺]=584

Example 6

Cyclo{-Suc-Trp(Me)-Phe-[(R)-NH-CH(CH₂C₆H₅)-CH₂-NH-]}

- (compound of formula I, in which R₁ = -CH₂-(N-methylindol-3-yl), and the other
substituents are as defined in Example 1) MS(ES⁺):[MH⁺]= 580

20 Example 7

Cyclo{-Suc-Phe(3,4-Cl)-Phe-[(R)-NH-CH(CH₂C₆H₅)-CH₂-NH-]}

- (compound of formula I, in which R₁ = -(3,4-dichlorobenzyl), and the other
substituents are as defined in Example 1) MS(ES⁺):[MH⁺]=595

Example 8

- 25 Cyclo{-Suc-Trp-Phe(3,4-Cl)-[(R)-NH-CH(CH₂C₆H₅)-CH₂-NH-]}

(compound of formula I, in which R₂ = -(3,4-dichlorobenzyl), and the other
substituents are as defined in Example 1) (MS(ES⁺):[MH⁺]= 634

Example 9

Cyclo{-Suc-Trp-Tyr-[(R)-NH-CH(CH₂C₆H₅)-CH₂-NH-]}

- 30 (compound of formula I, in which R₂ = -(4-hydroxybenzyl), and the other
substituents are as defined in Example 1) (MS(ES⁺):[MH⁺]= 582

Example 10

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂C₆H₃-3,4-diCl)-CH₂-NH-]}

(compound of formula I, in which R₃ = -(3,4-dichlorobenzyl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 634

Example 11

5 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂C₆H₄-4-OH)-CH₂-NH-]}

(compound of formula I, in which R₃ = -(4-hydroxybenzyl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 582

Example 12

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂-CH₂-C₆H₅)-CH₂-NH-]}

10 (compound of formula I, in which R₃ = -CH₂-CH₂-C₆H₅, and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 580

Example 13

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂-2-naphthyl)-CH₂-NH-]}

15 (compound of formula I, in which R₃ = -CH₂-(2-naphthyl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 616

Example 14

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂-indol-3-yl)-CH₂-NH-]}

(compound of formula I, in which R₃ = -CH₂-(indol-3-yl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 605

20 Example 15

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂-5-F-indol-3-yl)-CH₂-NH-]}

(compound of formula I, in which R₃ = -CH₂-(5-fluoroindol-3-yl), and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 623

Example 16

25 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂C₆H₄-3-F)-CH₂-NH-]}

(compound of formula I, in which R₃ = -CH₂C₆H₄-3-F, and the other substituents are as defined in Example 1) (MS(ES+):[MH+]= 584

Example 17

Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₃-3,4-diF)-CH₂-NH-]}

30 (compound of formula I) wherein R₃ = -(3,4-difluorobenzyl) and the other substituents are as defined in Example 1 MS (ES+): [MH+]= 602

Example 18

Cyclo{-Suc-Trp-Phe-[(R)NH-CH(CH₂-C₆H₄-4-CF₃-CH₂-NH)-]}

(compound of formula (I) wherein R₃ = -(4-trifluoromethylbenzyl) and the other substituents are as defined in Example 1) MS (ES⁺): [MH⁺]= 634

Example 19

5 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH₂-CH(CH₂C₆H₅)-NH-]}

(compound of formula (I) where: X₁ = X₂ = X₃ = X₄ = -CO-NH-; R₁ = -CH₂-(indol-3-yl); R₂ = R₃ = -CH₂-C₆H₅; R₄ = R₅ = R₆ = R₇ = H; f = h = 0; m = g = 1; the carbon atoms C-R₁ and C-R₂ have an S configuration, whereas C-R₃ has an R configuration)

10 a) Synthesis of (R)-2-*tert*-butoxycarbonylamino-3-phenyl-propylamine

The synthesis was performed according to the method described by P.G. Mattingly, Synthesis, 1990, 366, starting from BOC-D-phenylalaninol.

b) Synthesis of Z-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-NH-BOC] (5)

(R)-2-*tert*-butoxycarbonylamino-3-phenyl-propylamine (titre 65%, 1.1 g), PyBOP (1.45 g), and DIEA (0.98 ml) were added to a solution of Z-Trp-Phe-OH (1.4 g) in anhydrous DMF (15 ml) under nitrogen. The reaction mixture was kept stirred overnight at room temperature, AcOEt (100 ml) was added, and the mixture was washed with HCl 1N (3 x 30 ml), Na₂CO₃ 5% (3 x 30 ml), and H₂O (30 ml). During the washings, the product partly precipitated, and was filtered and re-
15 united to the organic phase. After vacuum evaporation of the solvent, the residue (2.4 g) was washed in suspension with AcOEt and vacuum dried on P₂O₅, to obtain 1.79 g of the desired compound 5 as a white solid.

TLC: r.f. 0.86 (CHCl₃/MeOH = 95/5); r.f. 0.78 (AcOEt)

c) Synthesis of H-Trp-Phe-[(R)-NH-CH₂-CH(CH₂-C₆H₅)-NH-BOC] (6)

25 A suspension of the compound 5 (1.7 g) in MeOH (350 ml) was hydrogenated in the presence of Pd/C 10%, at atmospheric pressure and room temperature, until the precursor disappeared (HPLC analysis). After elimination of the catalyst by filtration and vacuum evaporation of the solvent, the residue was washed in suspension with AcOEt to obtain 890 mg of the desired compound 6
30 as a white solid.

TLC: r.f. 0.38 (CHCl₃/MeOH = 9/1), r.f. 0.26 (AcOEt)

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d) Synthesis of HO-Suc-Trp-Phe-[(R)-NH-CH₂-CH(CH₂-C₆H₅)-NH-BOC]
(compound 8 where: PG₂ = OH; PG₁ = BOC)

Succinic anhydride (158 mg) and NEt₃ (0.21 ml) were added to a solution of compound 6 (840 mg) in anhydrous DMF (20 ml) under nitrogen. The reaction mixture was kept stirred at room temperature overnight. After vacuum evaporation of the solvent at a temperature of 30°C, the residue was treated with H₂O at 40-50°C, filtered, washed in suspension with MeOH (15 ml), and vacuum dried to obtain 600 mg of the desired compound 8 as a white solid.

TLC: 0.63 (CHCl₃/MeOH = 8/2)

e) Synthesis of HO-Suc-Trp-Phe-[(R)-NH-CH₂-CH(CH₂-C₆H₅)-NH₂]• TFA (9 TFA)

TFA (2ml) was added, under stirring, to a suspension of compound 8 (560 mg) in CH₂Cl₂ (15 ml), and a clear solution was obtained. After 2 hours at room temperature, the solvent was evaporated, and the residue diluted with ether, filtered and dried to obtain 500 mg of the desired compound 9 TFA as an ivory-coloured solid.

TLC: 0.58 (CHCl₃/MeOH = 8/2), 0.74 (*n*-butanol/AcOH/H₂O = 6/2/2)

f) Synthesis of cyclo{-Suc-Trp-Phe-[(R)-NH-CH₂-CH(CH₂-C₆H₅)-NH-]} (10)

PyBOP (447 mg), and DIEA (0.37 ml) were added, under nitrogen, to a solution of 9 TFA (500 mg) in anhydrous DMF (20 ml). The reaction mixture was kept stirred overnight at room temperature. After evaporation of the solvent, the residue was washed in suspension with citric acid 5% and H₂O. The product was dried on P₂O₅, washed in suspension using AcOEt and MeOH under heat, to obtain 110 mg of a solid. A portion was purified by HPLC to obtain 25 mg of the desired compound 10 as a white solid.

¹H-NMR (DMSO): δ 2.10-2.40 (m, 4H), 2.45-2.58 (m, 1H), 2.60-3.05 (m, 7H), 3.80-3.90 (m, 1H), 3.92-4.05 (m, 1H), 4.20-4.38 (m, 1H), 6.90-7.40 (m, 16H), 7.52-7.58 (m, 1H), 8.11 (d, 1H), 8.37 (d, 1H), 10.79 (s, 1H).

Example 20

Cyclo{-Suc-Trp-Phe-[(S)-NH-CH₂-CH(CH₂-C₆H₅)-NH-]}

(compound of formula I in which the substituents are defined as in Example 19, except for the fact that C-R₃ has an S configuration).

The compound was obtained following a procedure similar to that described for Example 19.

¹H-NMR (DMSO): δ 1.98-2.26 (m, 2H), 2.40-2.88 (m, 8H), 2.98-3.11 (m, 1H), 3.66-3.84 (m, 1H), 3.98-4.23 (m, 2H), 4.40-4.58 (m, 1H), 6.89-7.48 (m, 17H), 8.10 (d, 1H), 8.44 (d, 1H), 10.83 (s, 1H).

Proceeding in a similar way as that described in Example 1 above, the following compounds were obtained:

Example 21

Cyclo{-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH-]-(CH₂)₃CO-}

(compound of formula I, in which R₃ = -CH₂-C₆H₅ and X₃ = -CH₂-NH-, and the other substituents are as defined in Example 1. MS (ES⁺): [MH⁺]=552.

Example 22

Cyclo{-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-N(CH₃)]-(CH₂)₃CO-}

(compound of formula I, wherein R₃ = -CH₂-C₆H₅ and X₃ = -CH₂N(CH₃)- and the other substituents are as defined in Example 1. MS(ES⁺):[MH⁺]=566.

EXAMPLE 23

Cyclo{-Suc[1(S)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}

(compound of formula I wherein h = 1, g = 0, R₄ = -NH₂ and the other substituents are as defined in Example 1 while the carbon atom C-R₄ has configuration S).

a) Synthesis of Boc-Asp[Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂-NH-Z]-OBz]

(compound 8 wherein: PG₂ = OBzl, PG₁ = Z

To a solution of compound 6 (see Example 1d) (650 mg) in anhydrous DMF (30 ml) Boc-Asp-OBzl (340 mg), PyBOP (656 mg) and ET₃N (0.4 ml) are added under stirring at room temperature. The mixture is stirred for 2 h at room temperature. After evaporation of the solvent under vacuum the residue was treated with H₂O giving a solid residue which is filtered, washed with water and dried. The solid was recrystallized from ethanole giving 640 mg of the desired compound 8 in the form of a white solid.

MS (ES⁺): [MH⁺]=923; HPLC performed in the following conditions: silica column C₁₈ particles size 5μm and pores of 100 Å (analytical data: 20% carbon and C₁₈ Surface Coverage 3.3 μmoles/m²), length: 3.9x150mm; mobile phase

having a linear gradient of acetonitrile containing 0.1%(v/v) TFA (phase B) against aqueous TFA 0.1% (v/v) (phase A), from 20% to 80% in B in 20 minutes at a flux of 3 ml/min; determination by UV at 220 nm. Retention time: $R_t = 21.1$ min.

5 b) Synthesis of Boc-Asp[Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂-NH₂]]-OH (9)

The compound 8 (of Example 23a) (600 mg) was solved in DMF (2 ml) and diluted with MeOH (30 ml), hydrogenated in the presence of Pd/C 10% (100 mg) at room pressure and temperature for 5 h. The catalyser was filtered and washed with MeOH. After evaporation of the solvent 420 mg of the desired product 9 were obtained in the form of a white solid.

MS(ES⁺):[MH⁺] = 663; HPLC (same conditions as above): $R_t = 11.07$.

c) Synthesis of cyclo{-Suc[1(S)NH-BOC]-Trp-Phe[(R)NH-CH(CH₂-C₆H₅)-CH₂-NH]-} (10)

To a solution of compound 9 (see example 23b) (7.2 g) in anhydrous DMF (900 ml) 4 g of HBT and 2 g of EDC were added under stirring and nitrogen atmosphere. The mixture was stirred for 5 h and, after evaporation of the solvent, the residue was treated with an aqueous solution of KHSO₄ 5% and extracted in ethylacetate.

The organic phase was washed with brine, NaHCO₃ 5% and again with brine, dried and evaporated the yellow solid obtained (5.2 g) was crystallized from isopropanol/water: 1/1 giving 3.2 g of a white solid. MS(ES⁺):[MH⁺] = 681; HPLC (same conditions as above): $R_t = 14.8$.

d) Synthesis of cyclo{-Suc[(1(S)NH₂)-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂-NH]]} (10)

To a suspension of compound 10 (see example 23d) (1g) in CH₂Cl₂ (20 ml) TFA (7 ml) was added under stirring at 0°C giving a clear solution; thereafter the temperature is raised up to room temperature. The mixture was left at room temperature for 90 minutes and then the solvent was evaporated and the residue was treated with NaHCO₃ and water and extracted in ethylacetate. The organic phase was washed with brine, dried and evaporated giving a solid (800 mg).

MS(ES⁺):[MH⁺] = 581; HPLC (same conditions as above said): $R_t = 9.4$.

A sample of 20 mg is purified by HPLC giving 15 mg of trifluoroacetate: cyclo{-Suc[1(S)NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂-NH]-}.TFA (10 TFA)

MS(ES⁺):[MH⁺]= 581; HPLC: Rt= 9.4 (same conditions as above); ¹H-NMR (DMSO): δ 2.60-2.90 (m, 8H), 3.05-3.11 (m, 1H), 3.63-3.71 (m, 1H), 4.07-4.13 (m, 3H), 4.32-4.38 (m, 1H), 6.90-7.45 (m, 17H), 8.07 (bs, NH₃⁺), 8.22-8.28 (m, 1H), 8.57 (d, 1H), 10.82 (s, 1H).

Following the procedure described in Example 23 the following compounds were obtained:

Example 24

- 10 Cyclo{-Suc[1(R)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
(compound of formula I wherein h = 1, g = 0, R₄ = -NH₂ and the other substituents are as defined in Example 1 while the carbon atom C-R₄ has configuration R) MS (ES⁺): [MH⁺] = 581

Example 25

- 15 Cyclo{-Suc[2(S)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
(compound of formula I wherein h = 1, g = 0, R₄ = -NH₂ and the other substituents are as defined in Example 1 while the carbon atom C-R₄ has configuration S) MS (ES⁺): [MH⁺] = 581

Example 26

- 20 Cyclo{-Suc[2(R)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
(compound of formula I wherein h = 1, g = 0, R₄ = -NH₂ and the other substituents are as defined in Example 1 while the carbon atom C-R₄ has configuration R) MS (ES⁺): [MH⁺] = 581

Example 27

- 25 Cyclo{-Suc[1(S)-NH(CH₃)]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
(compound of formula I wherein h = 1, g = 0, R₄ = -NH(CH₃) and the other substituents are as defined in Example 1 while the carbon atom C-R₄ has configuration S) MS (ES⁺): [MH⁺] = 595

Example 28

- 30 Cyclo{-Suc[1-COO(CH₂-C₆H₄-4-NO₂)]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}

(compound of formula I wherein $h = 1$, $g = 0$, $R_4 = -\text{COO}(\text{CH}_2-\text{C}_6\text{H}_4-4-\text{NO}_2)$ and the other substituents are as defined in example 23) (diastereoisomeric mixture in respect of C- R_4 and separation of the two epimers).

a) Synthesis of Boc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH₂]

- 5 The Boc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH-Z] (5 see example 1c) (1.2 g) was dissolved in a mixture of DMF (30 ml) and MeOH (200 ml) and hydrogenized in the presence of Pd/C 10% (200 mg) at room pressure and temperature, for 4 h. The catalyser was filtered and washed with MeOH, the solvent evaporated giving 700 mg of solid residue.

- 10 MS(ES⁺): [MH⁺] 584; HPLC (conditions of example 23): Rt = 11.1

b) Boc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-COCH[COO(CH₂-C₆H₄-4-NO₂)]CH₂COO-tBu

- 424 mg of 2-(4-nitro-benzyloxycarbonyl)-succinic acid 4-tert-butyl ester were dissolved in DMF (20 ml). To the mixture HOBt (490 mg), EDC and Boc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH₂] were added at 0°C under stirring; the temperature was raised to room temperature while stirring for 2 h. The solvent was evaporated and the residue treated with KHSO₄ 5% giving a yellow solid which was filtered, washed with NaHCO₃ 5%, water and dried.

- 1.05 g of compound were obtained, MS(ES⁺):[MH⁺] = 919; HPLC (conditions of Example 23): Rt = 20.36

c) H-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-COCH[COO(CH₂-C₆H₄-4-NO₂)]CH₂COOH

- Boc-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-COCH[COO(CH₂-C₆H₄-4-NO₂)]CH₂COO-tBu (1.05 g) was added in small portions in anhydrous trifluoroacetic acid (20 ml) at 0°C and the mixture was kept under stirring for 30 minutes, dried and the residue treated with ethyleter; the formed solid was filtered, washed with ethyleter and dried, 850 mg of product were obtained.

MS(ES⁺):[MH⁺] = 763; HPLC (conditions of example 23): Rt = 10.6

- d) Synthesis of cyclo{-Suc[1-COO(CH₂C₆H₄-4-NO₂)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH-]}

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The H-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-COCH[COO(CH₂C₆H₄-4-NO₂)]CH₂COOH (100 mg) was dissolved in DMF (5 ml) and to the mixture PyBOP (80 mg) and Et₃N (54 µl) were added stirring for 3 h.

The reaction mixture was dried and the residue dissolved in ethylacetate, the organic phase was washed with KHSO₄ 5%, brine, NaHCO₃ 5% and brine, dried and concentrated. 90 mg of epimeric mixture was obtained, the epimers were separated by HPLC giving:

30 mg of liophylized solid which in HPLC (conditions of example 23) shows an Rt = 15.2. MS(ES+):[MH+]= 745.

¹H-NMR (DMSO): δ 2.54-2.81 (m, 7H), 3.08-3.17 (m, 1H), 3.34-3.39 (m, 1H), 3.77-3.84 (m, 1H), 4.00-4.10 (m, 3H), 4.37-4.43 (m, 1H), 5.31 (s, 2H), 6.60 (d, 1H), 6.93-7.42 (m, 16H), 7.62 (d, 2H), 8.24 (d, 2H), 8.60 (d, 1H), 8.66-8.72 (m, 1H), 10.81 (s, 1H) and

7 mg of liophylized solid which in HPLC (conditions of example 23) shows an Rt = 15.7. MS(ES+): [MH+]= 745.

Example 29

Cyclo{-Suc(1-COOH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]} (compound of formula I, wherein h = 1, g = 0, R₄ = -COOH and the other substituents are as defined in Example 1) [epimer which in HPLC (conditions of Example 23) shows an Rt = 10.7]

The cyclo{-Suc(1-COO(CH₂-C₆H₄-4-NO₂))-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]} which in HPLC (same conditions as in example 23) shows an Rt = 15.2 (50 mg) was suspended in a mixture water/isopropanol:1/1 (6 ml) containing K₂CO₃ (19 mg) and was kept under stirring for 24 h. The solvent was evaporated and the residue was diluted with water and the solution washed with ethylacetate, by adding HCl 1N separated a solid which was extracted with ethylacetate; the organic phase was washed with brine and dried. By evaporating the solvent mg 35 of a solid residue were obtained.

MS(ES+):[MH+]=610. HPLC (conditions of Example 23): Rt = 10.7

Example 30

Cyclo{-Suc(1-COOH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]} (compound of formula I, wherein h = 1, g = 0, R₄ = -COOH and the other substituents are as

defined in Example 1) [epimer which in HPLC (conditions as in Example 23) shows an $R_t = 11.1$]

The cyclo{-Suc(1-COO(CH₂-C₆H₄-4-NO₂))-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]} having $R_t = 15.7$ was hydrolyzed as described in Example 29.

- 5 MS(ES⁺):[MH⁺]= 610; HPLC (same conditions of Example 23): $R_t = 11.1$

As described in Example 28 the following compounds were obtained :

Example 31

Cyclo{-Suc(1-OH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]}

- 10 (compound of formula I, in which $h = 1$; $g = 0$; $R_4 = -OH$, and the other substituents are as defined in Example 1), MS(ES⁺):[MH⁺]= 582.

Example 32

Cyclo{-Suc(2-COOH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]}

(compound of formula I, in which $R_4 = -COOH$, and the other substituents are as defined in Example 1) MS(ES⁺):[MH⁺]: 610.

- 15 Example 33

Cyclo{-Suc(2-OH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]} (compound of formula I wherein: $h = 0$, $g = 1$, $R_4 = OH$ and the other substituents are as defined in example 1) MS(ES⁺): [MH⁺] = 582.

- 20 The compounds of Examples 23, 24, 25, 26, 27, 29, 30 and 32 can be derivatized as described hereinafter.

Example 34

- 25 Cyclo{-Suc[1(S)-(2H-tetrazolyl-5-ylmethyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}.TFA (compound of formula I wherein $h = 1$, $g = 0$, $R_4 = -(2H-tetrazolyl-5-ylmethyl)amino$ and the other substituents are as defined in example 1 while the carbon atom C- R_4 has configuration S)

a) Synthesis of 5-iodomethyl-1-trityl-1H-tetrazole

- 30 To a suspension of 5-chloromethyl-1H-tetrazole (6.0 g) in chloroform (100 ml) trityl-chloride (14.2 g) was added at 0°C under nitrogen, and the mixture was stirred up to total solubilization, thereafter a solution of Et₃N (7.0 ml) in chloroform (50 ml) was added at 5°C and the temperature was left raising up to room temperature, the mixture was kept resting for 24 h.

The mixture was treated with ethylacetate (200 ml) and left resting for 6h, the separated solid was filtered away and to the solution acetone (70 ml) was added, the precipitated solid was collected by filtration and dried giving 9.5 g of 5-chloromethyl-1-trityl-1H-tetrazole which was solubilized in acetone (200 ml) at 60°C. Sodium iodide (5.6 g) was added to the solution which was refluxed for 6 h, by cooling precipitated a compound which was filtered, washed with water and dried giving 5.2 g of a white solid.

TLC: R.f. 0.55 (AcOEt/Cyclohexane : 1/3)

b) Synthesis of cyclo{-Suc[1(S)-(2-trityl-tetrazolyl-5-ylmethyl)amino]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}

To 205 mg of cyclo{-Suc[1(S)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-} (compound of example 23) in anhydrous DMF (5 ml) were added, under stirring 5-iodomethyl-1-trityl-1H-tetrazole (147 mg) and thereafter DIEA (0.06 ml) keeping the temperature at 0°C for 4 h and at room temperature for 3 h. The mixture was treated with water and extracted with ethylacetate, the organic phase was washed with brine and dried. By evaporating the solvent a solid was obtained which was purified by column-chromatography eluting with AcOEt/MeOH = 95/5. 210 mg of product were obtained. MS(ES⁺):[MH⁺]=905; HPLC (conditions of example 23): Rt=15.4.

c) synthesis of cyclo{-Suc[1(S)-(2-HI-tetrazolyl-5-ylmethyl)amino]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}.TFA

To a solution of cyclo{-Suc[1(S)-(2-trityl-tetrazolyl-5-ylmethyl)amino]Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-} (90 mg) in anhydrous DMF (5 ml) a solution of HCl 4M in dioxane (0.6 ml) was added at 0°-5°C, the temperature was brought to room temperature and the mixture was left resting up to end of the reaction (14 h at room temperature and 56 h at 5°C) checking the reaction by HPLC. The solvent was evaporated and the residue treated with AcOEt, the organic phase was washed with brine and dried; evaporating the solvent 30 g of a crude solid are obtained, the solid is purified by HPLC giving 10 g of liophilized solid product.

MS(ES⁺):[MH⁺]=663; HPLC (conditions of example 23): Rt=9.0

1H-NMR (DMSO): δ 2.62-2.92 (m, 8H), 3.16-3.23 (m, 1H), 3.68-3.74 (m, 1H), 4.00-4.14 (m, 3H), 4.25-4.75 (m, 3H), 6.88-7.42 (m, 17H), 8.30-8.37 (m, 1H), 8.54 (d, 1H), 10.82 (s, 1H).

Example 35

- 5 Cyclo{-Suc[1(S)-(morpholin-4-yl)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R₄ = -(morpholin-4-yl) and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

To a solution of 2,2'-oxydiacetaldehyde (1mmole), excess, in methanole (20 ml)
10 58 mg of cyclo{-Suc[1(S)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-} (compound of example 23), 0.2 ml of acetic acid and 12 mg of NaCNBH₃ were added. After 2 h the mixture was diluted with water (10 ml), treated with HCl 1N up to pH 3 and the methanole was evaporated; the solution was treated with NaHCO₃ 5% and the formed solid was extracted with ethylacetate. The organic
15 phase, after washing with brine and anhydrification, was evaporated giving 58 mg of a solid which was purified by HPLC giving 10 mg of liophylized solid trifluoroacetate.

MS(ES⁺):[MH⁺]=651; TLC: R.f.0.20 (CHCl₃/MeOH:9/1)

1H-NMR (DMSO): δ 2.62-3.00 (m, 8H), 3.27-3.87 (m, 10H), 4.07-4.15 (m, 3H),
20 4.32-4.38 (m, 1H), 6.62 (d, 1H), 6.94-7.41 (m, 16H), 8.49-8.64 (m, 2H), 10.84 (s, 1H).

Via a similar reductive amination reaction, as described in example 35, the following compounds were obtained:

Example 36

- 25 Cyclo{-Suc[1(S)-N(CH₃)₂]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R₄ = -N(CH₃)₂ and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

The synthesis was performed starting from the compound of example 23 using
30 paraformaldehyde. MS(ES⁺):[MH⁺]=609.

Example 37

Cyclo{-Suc[1(S)-(piperidin-4-yl)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R₄ = -(piperidin-4-yl) and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

- 5 The synthesis was performed starting from the compound of example 23 using glutaraldehyde. MS(ES+):[MH+]=649.

Example 38

- Cyclo{-Suc[1(S)-(N(CH₂CH₂OH)₂)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R₄ = -N(CH₂CH₂OH)₂ and
10 the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

The synthesis was performed starting from the compound of example 23 using glycolaldehyde. MS(ES+):[MH+]=669.

Example 39

- 15 Cyclo{-Suc[1(S)-(NHCH₂CH(OH)CH₂OH)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}.TFA (compound of formula I wherein h = 1, g = 0, R₄ = -NHCH₂CH(OH)CH₂OH and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

- The synthesis was performed starting from the compound of example 24 using
20 D-glyceraldehyde. MS(ES+):[MH+]=655.

Example 40

- Cyclo{-Suc[1(S)-(3-carboxypropanoyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}. (compound of formula I wherein h = 1, g = 0, R₄ = -(3-carboxypropanoyl)amino and the other substituents are as defined in example
25 1 while the carbon atom C-R₄ has configuration S)

- To a solution of the compound of Example 23 (100 mg) in anhydrous DMF (2 ml) succinic anhydride (30 mg) and dimethylamino-pyridine (10 mg) were added and the solution was stirred for 16 h; the solvent was evaporated giving a solid which was solubilized in ethylacetate, washed with citric acid 10%, brine and
30 dried. By evaporating the solvent a solid compound was collected (90 mg), which purified by HPLC gave 60 mg of a lyophilized solid.

MS(ES+):[MH+]=681; HPLC (conditions as in example 23): Rt=10.8

1H-NMR (DMSO): δ 2.35-2.82 (m, 12H), 3.25-3.28 (m, 1H), 3.66-3.73 (m, 1H), 3.98-4.12 (m, 2H), 4.33-4.38 (m, 1H), 4.67-4.73 (m, 1H), 6.80 (d, 1H), 6.96-7.39 (m, 16H), 8.16-8.23 (m, 2H), 8.51 (d, 1H), 10.89 (s, 1H).

Example 41

- 5 Cyclo{-Suc[1(S)-[3-N'-(β -D-glucopiranos-1-yl)-carboxamidopropanoyl]amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} (compound of formula I wherein h = 1, g = 0, R₄ = -[3-N'-(β -D-glucopiranos-1-yl)carboxyamidopropanoyl]amine] and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

- 10 The compound of example 40 (90 mg) was dissolved in anhydrous DMF (10 ml) under stirring and in nitrogen atmosphere, to the mixture 55 mg HBT, 25 mg EDC and 24 mg β -D-glucopiranosylamine were added.

- The mixture was left stirring overnight and after evaporation of the solvent the resulting oil was treated with citric acid 10% giving a solid which was filtered,
15 washed with water and dried. The 80 mg obtained were purified by HPLC giving 40 mg of a lyophilized solid.

MS(ES⁺):[MH⁺] = 842; HPLC (conditions of example 23): Rt = 8.2

- 1H-NMR (DMSO): δ 2.31-2.81 (m, 12H), 3.00-3.10 (m, 2H), 3.13-3.65 (m, 5H), 3.66-3.75 (m, 1H), 3.97-4.12 (m, 2H), 4.29-4.36 (m, 1H), 4.65-4.75 (m, 2H), 6.78
20 (d, 1H), 6.95-7.40 (m, 16H), 8.19-8.27 (m, 2H), 8.35 (d, 1H), 8.51 (d, 1H), 10.82 (s, 1H).

Example 42

- Cyclo{-Suc[1(S)-[(carboxymethyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} TFA (compound of formula I wherein h = 1, g = 0, R₄ = -
25 (carboxymethyl)amino and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

a) Synthesis of cyclo{-Suc[1(S)-[(ter-butoxycarbonylmethyl)-amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}

- To a solution of the compound of example 23 (130 mg) in anhydrous DMF (3
30 ml) DIEA (0.04 ml) and ter-butyle (0.04 ml) bromoacetate were added, the solution was stirred for 2 h and thereafter the mixture was poured in KHSO₄

5%. The formed solid was filtered, washed with NaHCO_3 , water and dried. 100 mg of product were obtained.

HPLC (conditions of Example 23): $R_t = 11.3$

- b) Synthesis of cyclo{-Suc[1(S)-[(carboxymethyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}] .TFA

The above collected solid (90 mg) was suspended in CH_2Cl_2 (5 ml) and TFA (5 ml) was added under stirring at 0°C, the mixture was stirred for 1 h at room temperature. The solution was concentrated and the obtained residue was purified by HPLC giving 40 mg of a lyophilized solid.

- 10 MS(ES⁺):[MH⁺]=639; HPLC (conditions of Example 23): $R_t=9.4$.

Example 43

- Cyclo{-Suc[1(S)-[N'-(β-D-glucopiranos-1-yl)-carboxyamidomethyl]amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} TFA (compound of formula I wherein h = 1, g = 0, R₄ = -[N'-(β-D-glucopiranos-1-yl)carboxyamidomethyl]amine] and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

The product was obtained starting from the product of Example 42 and β-D-glucopiranosylamine according to the procedure of Example 41.

MS(ES⁺):[MH⁺]=800; HPLC (conditions of example 23): $R_t= 7.6$

- 20 Example 44

cyclo{-Suc[1(S)-(chiny)amine]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} (compound of formula I wherein h = 1, g = 0, R₄ = -(chiny)amine and the other substituents are as defined in example 1. while the carbon atom C-R₄ has configuration S)

- 25 Chinic acid (50 mg) was solubilized in anhydrous DMF (10 ml) under stirring and nitrogen atmosphere, HBT (220 mg), EDC (100 mg) and the compound obtained in Example 24 (150 mg) were added. The mixture was left under stirring overnight, thereafter the solvent was evaporated and the residue treated with an aqueous solution of KHSO_4 5% and extracted with ethylacetate.

- 30 The organic phase was washed with brine, NaHCO_3 5% and again brine, dried and evaporated; the obtained solid (122 mg) was purified on flash

chromatography (SiO₂) eluting with chloroform/methanol:8/2; 80 mg of the desired compound were obtained.

MS(ES⁺):[MH⁺]=755; HPLC (conditions as in example 23) Rt=10.05

Example 45

- 5 Cyclo{-Suc[1(S)-(4-aminobutanoyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} TFA (compound of formula I wherein h = 1, g = 0, R₄ = -(4-aminobutanoyl)amino and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

The product was obtained starting from the product of Example 23 and 4-BOC-aminobutyric acid according to the procedure of Example 44 followed by elimination of the protecting group BOC.

MS(ES⁺):[MH⁺]=666.

Example 46

- 15 Cyclo{-Suc[1(S)-[(1,4')bipiperidin-1-yl]acetamido]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} TFA (compound of formula I wherein h = 1, g = 0, R₄ = -[(1,4')bipiperidin-1-yl]acetamido and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

The product was obtained starting from the product of Example 23 and [(1,4')bipiperidin-1-yl]acetic acid according to the procedure of Example 44.

- 20 MS(ES⁺):[MH⁺]=789.

Example 47

- 25 Cyclo{-Suc[1-N-(β-D-glucopiranos-1-yl)-carboxyamido]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} (compound of formula I wherein h = 1, g = 0, R₄ = N-(β-D-glucopiranos-1-yl)carboxamide and the other substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

The product was obtained starting from the product of Example 29 and β-D-glucopiranosylamine according to the procedure of Example 44.

MS(ES⁺):[MH⁺]= 771.

Example 48

- 30 Cyclo{-Suc[1(S)-[N'-(2-N-acetyl-β-D-glucopiranos-1-yl)-carboxyamido]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} (compound of formula I wherein h = 1, g = 0, R₄ = -N'-(2-N-acetyl-β-D-glucopiranos-1-yl)carboxamide and the other

substituents are as defined in example 1 while the carbon atom C-R₄ has configuration S)

The product was obtained starting from the acid of Example 29 and 2-N-acetyl-β-D-glucopiranosylamine according to the procedure of Example 44.

5 MS(ES+):[MH+]=812.

Biological activity

The compounds described in the present invention act as antagonists of the NK-2 receptor of tachykinins. The biological activity was evaluated in two *in-vitro* functional tests, using rabbit pulmonary artery (RPA) and hamster trachea (HT), according to the methods described by C.A. Maggi *et al.*, Br. J. Pharmacol., 1990, 100, 588 and P. D'Orléans-Juste *et al.*, Eur. J. Pharmacol., 1986, 125, 37. The activity of the compounds as human NK-2 receptor antagonists was assessed in a binding test using membranes of Chinese hamster ovary (CHO) cells, transfected with the NK-2 receptor of human ileum and the radioligand [¹²⁵I]NKA (Amersham, specific activity 2000 Ci/mmol) at a concentration of 100 pM in competition studies. The substances under examination were tested in a concentration range of from 0.01 nM to 10 mM. At the end of incubation (30 minutes at 20°C), the samples were filtered on Whatman GF/B filters and employing the Brandel automatic filtration system.

20 Radio-activity was determined by means of a gamma counter (Cobra, Canberra Packard).

The data gathered from the functional studies were expressed as pA₂ (O. Arunlakshana and H.O. Schild, Br. J. Pharmacol. Chemother., 1959, 14, 48), and those of the binding studies as pKi (-log Ki calculated using the LIGAND programme: P.J. Munson *et al.*, Anal. Biochem., 1980, 107, 220).

25

The compounds of the invention proved active in the tests referred to above, with pA₂ values of between 5 and 9, the more powerful compounds revealing a higher affinity for the human receptor, with pKi of between 8 and 10.

List of abbreviations used

30 For the nomenclature and abbreviations of amino acids, reference is made to the recommendations of the IUPAC-IUB Joint Commission on Biochemical

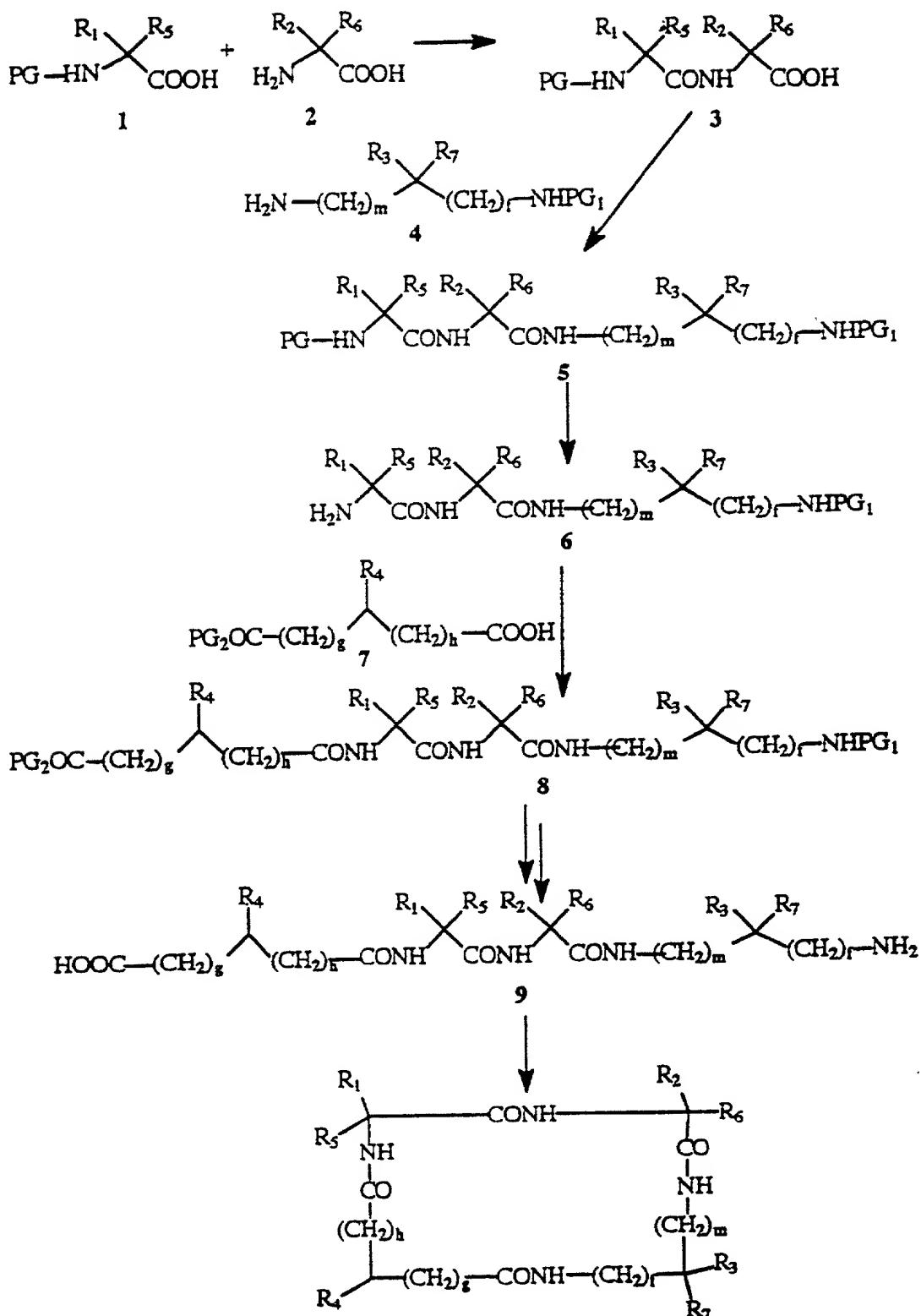
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Nomenclature (Eur. J. Biochem., 1984, 138, 9); the amino acids are understood in the S configuration, if not otherwise specified.

The other abbreviations used are the following:

- BOC = *tert*-butoxycarbonyl; Z = benzyloxycarbonyl; -Suc- = succinyl; Bzl = benzyl; PyBOP = (benzotriazol-1-yloxy)*tris*(pyrrolidine) phosphonium hexafluorophosphate, DIEA = N,N-diisopropylethylamine; NEt₃ = triethylamine; DMF = N,N-dimethylformamide; NKA = neurokinine A; TFA = trifluoro-acetic acid; HBT = 1-hydroxybenzotriazole; EDC = N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride.
- 10 The numeration of the substituents on the succinic-group is as follows:
- Suc(1-NH₂)- = -CO-CH(NH₂)-CH₂-CO-
- Suc(2-NH₂)- = -CO-CH₂-CH(NH₂)-CO-

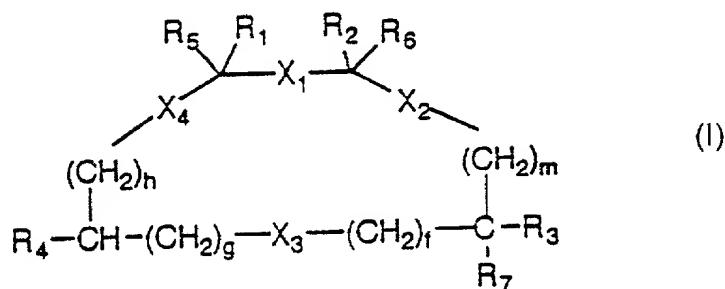
Scheme



where PG, PG₁ and PG₂ are protecting groups commonly used in the synthesis of peptides.

CLAIMS

1. Monocyclic compounds having the general formula (I):



in which:

X_1, X_2, X_3, X_4 , which may be the same or different from one another, represent a group chosen from among $-CONR-$, $-NRCO-$, $-OCO-$, $-COO-$, $-CH_2NR-$, $-NR-CH_2-$, CH_2-CH_2 , where R is H or a C_{1-3} alkyl or benzyl;

f, g, h, m, which may be the same or different from one another, represent a number chosen from among 0, 1 or 2;

R_1 and R_2 , which may be the same or different from one another, represent a $-(CH_2)_r-Ar$ group, where $r = 0, 1, 2$ and where Ar is an aromatic group chosen from among: benzene, naphthalene, thiophene, benzothiophene, pyridine, quinoline, indole, furan, benzofuran, thiazole, benzothiazole, imidazole, and benzo-imidazole, the said Ar group being possibly substituted with a maximum of 2 residues chosen from among C_{1-3} alkyl or halo-alkyl, C_{1-3} alkoxy, C_{2-4} amino-alkoxy, halogen, OH, NH_2 , $NR_{13}R_{14}$ where R_{13} and R_{14} , which may be the same or different from one another, represent hydrogen or C_{1-3} alkyl;

R_3 represents a group chosen from among:

- hydrogen

- linear or branched alkyl having the formula C_nH_{2n+1} , with $n = 1-5$, cyclo-alkyl or alkylcyclo-alkyl groups having the formula C_nH_{2n-1} with $n = 5-9$

- $(CH_2)_r-Ar_1$, where $r = 0, 1, 2$ and where Ar_1 is an aromatic group chosen from among: benzene, naphthalene, thiophene, benzothiophene, pyridine, quinoline, indole, furan, benzofuran, thiazole, benzothiazole, imidazole, and benzo-imidazole, the said Ar_1 group being possibly substituted with a maximum of 2 residues chosen from among C_{1-3} alkyl or halo-alkyl, C_{1-3} alkoxy or amino-alkoxy, halogen, OH, NH_2 , $NR_{13}R_{14}$, where R_{13} and R_{14} , which may be the same or different from one another, represent hydrogen or C_{1-3} alkyl;

- 32 R_4 represents a group chosen from among:
- 33 - hydrogen or C_{1-6} alkyl
- 34 - L-Q, where L is a chemical bond or a linear or branched C_{1-6} alkyl residue and
- 35 Q is a group chosen from among:
- 36 i) H, OH, OR_9 , NH_2 , NR_9R_{10} , guanidine, sulphate, phosphonate, phosphate,
- 37 where R_9 and R_{10} , which may be the same or different from one another,
- 38 represent a hydrogen, C_{1-3} alkyl group, C_{1-3} hydroxyalkyl, C_{1-3} dihydroxyalkyl, C_{1-3} alkyl- $CONHR_{12}$, C_{1-3} alkyltetrazole, C_{1-3} alkyl-COOH or wherein R_9R_{10} joined
- 39 together form with the N-atom a saturated 4-6 membered heterocycle possibly
- 40 containing a further heteroatom chosen in the group consisting of N, O, S and
- 41 wherein R_{12} is a mono-, di-, tri-glycosidic group possibly protected with one or
- 42 more C_{1-3} -acyl groups or substituted with amino-groups or C_{1-3} acylamino-
- 43 groups;
- 44 ii) COOH, tetrazole, SO_2NH_2 , $SO_2NHCOOR_8$, $CONHR_8$, $NHCOR_8$, where R_8
- 45 represents a linear or cyclic C_{1-6} alkyl chain containing one or more polar groups
- 46 chosen from among the group: OH, NH_2 , $NR_{15}R_{16}$, COOH, $CONHR_{12}$, PO_3H ,
- 47 SO_3H , OR_{11} and where R_{15} and R_{16} , which may be the same or different from
- 48 one another, represent a hydrogen or C_{1-3} alkyl group, and where R_{11} is a C_{1-3}
- 49 alkyl or C_{2-4} amino-alkyl chain, R_{12} is a mono-, di-, tri-glycosidic group possibly
- 50 protected with one or more C_{1-3} acyl groups or substituted with amino-groups or
- 51 C_{1-3} acylamino-groups or $R_{15}R_{16}$ joined together form with the N-atom a
- 52 saturated 4-6 membered heterocycle possibly substituted with C_{1-3} alkyl-groups
- 53 or with saturated 4-6 membered heterocycle-groups containing at least an N-
- 54 atom;
- 55 iii) $COOR_{17}$, $CONHR_{12}$, OR_{12} where R_{12} is a mono-, di- or tri-glycoside group
- 56 possibly protected with one or more C_{1-3} acyl groups or substituted with amine
- 57 or C_{1-3} acylamine groups and R_{17} is a group R_{12} as above defined or a group
- 58 C_{1-3} alkyl, C_{1-3} alkylphenyl, wherein the phenyl-group can be substituted with a
- 59 group OH, NO_2 , NH_2 , CN, CH_3 , Cl, Br;
- 60 R_5 , R_6 , R_7 , which may be the same or different from one another, represent a
- 61 hydrogen or C_{1-3} alkyl group; with the proviso that when R_1 and R_2 are benzyl
- 62

31a

63 or 4-hydroxybenzyl then R_3 and R_4 are not isopropyl, their pharmaceutically
64 acceptable salts, their enantiomers and mixture thereof.

- 1 2. Compounds according to Claim 1, in which:
2 f, g, h, m, which may be the same or different from one another, may be 0 or 1;
3 R_1 and R_2 , which may be the same or different from one another, represent the
4 side chain of a natural amino acid chosen from among tryptophan, phenyl
5 alanine, tyrosine, histidine or the side chain of a non-natural amino acid chosen
6 in the group:
7 tryptophan and phenyl alanine, either mono- or di-substituted with residues
8 chosen from among C_{1-3} alkyl or halo-alkyl, C_{1-3} alkoxy or amino-alkoxy,
9 halogen, OH, NH_2 , $NR_{13}R_{14}$, where R_{13} and R_{14} , which may be the same or
10 different from one another, represent a hydrogen or C_{1-3} alkyl group;
11 R_3 represents a group chosen from among:
12 - linear or branched alkyl having the formula C_nH_{2n+1} , with $n = 1-5$ (chosen in the
13 group consisting of methyl, ethyl, propyl, isopropyl, n-butyl, t-butyl) cycloalkyl or
14 alkylcycloalkyl of formula C_nH_{2n-1} with $n = 5-9$ (chosen in the group consisting of
15 cyclopentyl, cyclohexyl, methylcyclohexyl)
16 - $(CH_2)_r-Ar_1$, where $r = 1$ or 2 and where Ar_1 is an aromatic group chosen in the
17 group consisting of: α -naphthyl, β -naphthyl, phenyl, indole, the said Ar_1 group
18 being possibly substituted with a maximum of 2 residues chosen in the group
19 consisting of: C_{1-3} alkyl, CF_3 , C_{1-3} alkoxy, Cl, F, OH, NH_2 ;
20 R_4 represents an L-Q group where:
21 L is a chemical bond or CH_2 , and
22 Q is a group chosen from among:
23 - OH, NH_2 , NR_9R_{10} , OR_{11} , and where R_9 and R_{10} , which may be the same or
24 different from one another, represent a hydrogen or C_{1-3} alkyl group, C_{1-3} hydroxy
25 alkyl, C_{1-3} dihydroxyalkyl, C_{1-3} alkyl- $CONHR_{12}$ (wherein R_{12} is a monoglycosidic
26 group derived from D or L pentoses or hexoses (chosen in the group consisting
27 of ribose, arabinose, glucose, galactose, fructose, glucosamine, galactosamine
28 and their N-acetylated derivatives)), C_{1-3} alkyltetrazole, C_{1-3} alkyl-COOH or
29 wherein R_9R_{10} are joined together to form with the N atom a morpholine or a
30 piperidine ring and where R_{11} is a C_{1-3} alkyl chain, or a C_{2-4} amino-alkyl chain;

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- 31 - NHCOR_8 wherein R_8 is a cyclohexane containing from 2 to 4 OH groups, a C_{1-6}
 32 alkylchain containing a polar group (chosen in the group consisting of NH_2 ,
 33 COOH , CONHR_{12} (wherein R_{12} is as hereabove define) or [1,4']bipiperidine)
 34 - COOH , COOR_{17} or CONHR_{12} , wherein R_{12} is as hereabove defined and R_{17} is
 35 as R_{12} or a group 4-nitrobenzyl.
 36 - R_5 , R_6 , R_7 are H.

37 in which the carbon atom that carries the substituents R_3 and R_7 has
 38 configuration R.

1 3. Compounds according to Claim 2, as specified below:

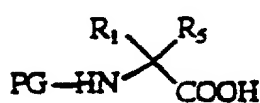
- 2 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_5$)- CH_2 -NH-]}
 3 Cyclo{-Suc-Trp-Phe-[(S)-NH-CH($\text{CH}_2\text{C}_6\text{H}_5$)- CH_2 -NH-]}
 4 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_{11}$)- CH_2 -NH-]}
 5 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_4$ (4-OCH₃))- CH_2 -NH-]}
 6 Cyclo{-Suc-Trp(5F)-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_5$)- CH_2 -NH-]}
 7 Cyclo{-Suc-Trp(Me)-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_5$)- CH_2 -NH-]}
 8 Cyclo{-Suc-Phe(3,4-Cl)-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_5$)- CH_2 -NH-]}
 9 Cyclo{-Suc-Trp-Phe(3,4-Cl)-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_5$)- CH_2 -NH-]}
 10 Cyclo{-Suc-Trp-Tyr-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_5$)- CH_2 -NH-]}
 11 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_3$ -3,4-diCl)- CH_2 -NH-]}
 12 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_4$ -4-OH)- CH_2 -NH-]}
 13 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{-CH}_2\text{-C}_6\text{H}_5$)- CH_2 -NH-]}
 14 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH_2 -2-naphthyl)- CH_2 -NH-]}
 15 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH_2 -indol-3-yl)- CH_2 -NH-]}
 16 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH(CH_2 -5-F-indol-3-yl)- CH_2 -NH-]}
 17 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{C}_6\text{H}_4$ -3-F)- CH_2 -NH-]}
 18 Cyclo{-Suc-Trp-Phe-[(R)NH-CH($\text{CH}_2\text{-C}_6\text{H}_3$ -3,4-diF)- CH_2 -NH-]}
 19 Cyclo{-Suc-Trp-Phe-[(R)NH-CH($\text{CH}_2\text{-C}_6\text{H}_4$ -4-CF₃)- CH_2 -NH-]}
 20 Cyclo{-Suc-Trp-Phe-[(R)-NH-CH₂-CH($\text{CH}_2\text{C}_6\text{H}_5$)-NH-]}
 21 Cyclo{-Suc-Trp-Phe-[(S)-NH-CH₂-CH($\text{CH}_2\text{C}_6\text{H}_5$)-NH-]}
 22 Cyclo{-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{-C}_6\text{H}_5$)- CH_2 -NH-]}-(CH_2)₃CO-}
 23 Cyclo{-Trp-Phe-[(R)-NH-CH($\text{CH}_2\text{-C}_6\text{H}_5$)- CH_2 -N(CH₃)]-(CH_2)₃CO-}
 24 Cyclo{-Suc[1(S)-NH₂]-Trp-Phe-[(R)NH-CH($\text{CH}_2\text{-C}_6\text{H}_5$)- CH_2 NH-]}

0955210.071200

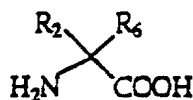
- 25 Cyclo{-Suc[1(R)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
- 26 Cyclo{-Suc[2(S)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
- 27 Cyclo{-Suc[2(R)-NH₂]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
- 28 Cyclo{-Suc[1(S)-NH(CH₃)]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
- 29 Cyclo{-Suc[1-COO(CH₂-C₆H₄-4-NO₂)]-Trp-Phe-[(R)NH-CH(CH₂-C₆H₅)-CH₂NH]-}
- 30 Cyclo{-Suc(1-COOH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]}
- 31 Cyclo{-Suc(1-COOH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]}
- 32 Cyclo{-Suc(1-OH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]}
- 33 Cyclo{-Suc(2-COOH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]}
- 34 Cyclo{-Suc(2-OH)-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]}
- 35 Cyclo{-Suc[1(S)-(2H-tetrazolyl-5-ylmethyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-
- 36 C₆H₅)-CH₂-NH]-}.TFA
- 37 Cyclo{-Suc[1(S)-(morpholin-4-yl)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-
- 38 }.TFA
- 39 Cyclo{-Suc[1(S)-N(CH₃)₂]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂NH]-}.TFA
- 40 Cyclo{-Suc[1(S)-(piperidin-4-yl)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂NH]-}.TFA
- 41 Cyclo{-Suc[1(S)-(N(CH₂CH₂OH)₂)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-
- 42 NH]}.TFA
- 43 Cyclo{-Suc[1(S)-(N(CH₂CH(OH)CH₂OH)]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-
- 44 NH]-}.TFA
- 45 Cyclo{-Suc[1(S)-(3-carboxypropanoyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-
- 46 CH₂-NH]-}.
- 47 Cyclo{-Suc[1(S)-[3-N'-(β-D-glucopiranos-1-yl)-carboxamidopropanoyl]amino]-
- 48 Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}
- 49 Cyclo{-Suc[1(S)-[(carboxymethyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-
- 50 NH]-} TFA
- 51 Cyclo{-Suc[1(S)-[N'-(β-D-glucopiranos-1-yl)-carboxyamidomethyl]amino]-Trp-
- 52 Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-} TFA
- 53 Cyclo{-Suc[1(S)-(chinyli)amine]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}
- 54 Cyclo{-Suc[1(S)-(4-aminobutanoyl)amino]-Trp-Phe-[(R)-NH-CH(CH₂-C₆H₅)-CH₂-
- 55 NH]-} TFA

- 56 Cyclo{-Suc[1(S)-[(1,4')bipiperidin-1-yl]acetamido]-Trp-Phe-[(R)-NH-CH(CH₂-
 57 C₆H₅)-CH₂-NH]-} TFA
 58 Cyclo{-Suc[1-N-(β-D-glucopiranos-1-yl)-carboxyamido]-Trp-Phe-[(R)-NH-
 59 CH(CH₂-C₆H₅)-CH₂-NH]-}
 60 Cyclo{-Suc[1(S)-[N'-(2-N-acetyl-β-D-glucopiranos-1-yl)-carboxyamido]-Trp-Phe-
 61 [(R)-NH-CH(CH₂-C₆H₅)-CH₂-NH]-}.

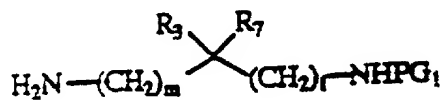
- 1 4. Process for the synthesis of a compound of general formula (I), where X₁, X₂,
 2 X₃, X₄ are CONH and the other substituents are as defined in Claim 1, where:
 3 a) the suitably protected amino acids (1), (2) and (4)



(1)

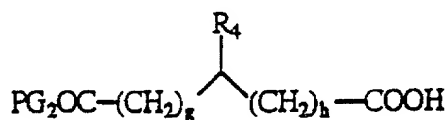


(2)



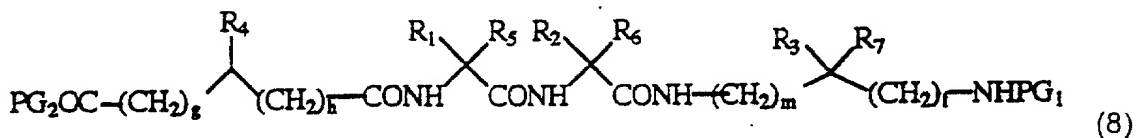
(4)

- 9 are made to react, as shown in the diagram, with the derivative of the protected
 10 succinic acid (7)

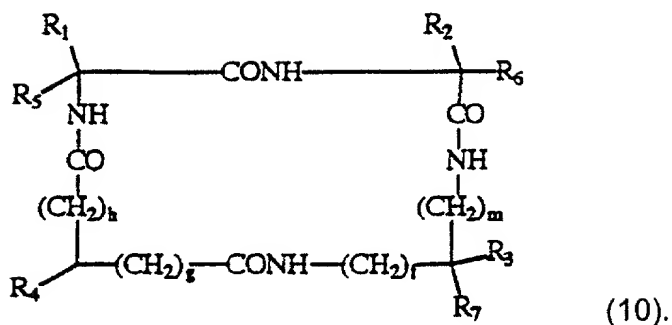


(7)

- 16 thus obtaining the linear compound (8)



- 21 b) the linear compound 8, is deprotected and cyclicized to yield the final
 22 monocyclic compound (10)



- 1 5. Pharmaceutical compositions containing as active principle the compounds
2 of general formula (I) according to Claim 1 in combination with pharmaceutically
3 acceptable carriers or excipients.
- 1 6. Pharmaceutical compositions according to Claim 5, to be used as tachykinin
2 antagonists.
- 1 7. Pharmaceutical compositions according to Claim 6, to be used as
2 antagonists of the human NK-2 receptor.
- 1 8. Pharmaceutical compositions according to Claim 7, to be used in the
2 treatment of the bronchospastic and inflammatory component of asthma,
3 coughing, pulmonary irritation, intestinal spasms, spasms of the biliary tract,
4 local spasms of the bladder and of the ureter during cystitis, and kidney
5 infections and colics.
- 1 9. Pharmaceutical compositions according to Claim 7, to be used as anxiolytics.
- 1 10. Use of a compound according to Claim 1 as tachykinin antagonist.
- 1 11. Use of a compound according to Claim 1 as NK-2 antagonist.
- 1 12. Use of a compound according to Claim 1 in the treatment of the
2 bronchospastic and inflammatory component of asthma, coughing, pulmonary
3 irritation, intestinal spasms, spasms of the biliary tract, local spasms of the
4 bladder and of the ureter during cystitis, and kidney infections and colics.
- 1 13. Use of a composition according to Claim 1 as an NK-2 antagonist for the
2 treatment of anxiety syndromes.

- 1 14. Method for the treatment of the bronchospastic and inflammatory
- 2 component of asthma, coughing, pulmonary irritation, intestinal spasms,
- 3 spasms of the biliary tract, local spasms of the bladder and of the ureter during
- 4 cystitis, and kidney infections and colics, in which quantities of between 0.02
- 5 and 10 mg/kg of body weight of active principle consisting of products of
- 6 formula (I), according to Claim 1, are administered to the patient.

0021/01012660

Docket No: 515-4167

**APPLICATION FOR UNITED STATES LETTERS PATENT
DECLARATION, POWER OF ATTORNEY, AND PETITION**

As a below-named inventor, I declare that:

My residence, post office address and citizenship are as stated next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the invention which is described and which is claimed in the specification, entitled: MONOCYCLIC COMPOUNDS WITH FOUR BIFUNCTIONAL RESIDUES HAVING NK-2 ANTAGONIST ACTION

The specification [] is attached hereto [X] was filed on 23 July 1999, as Application Serial No. 09/355,210.

☒ was filed as PCT international application
Number PCT/EP98/00599
on 4 February 1998
and was amended under PCT Article 19
on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of said specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.¹

COUNTRY	APPLICATION NUMBER	DATE (Day, Month, Year)	PRIORITY CLAIMED UNDER 35 U.S.C. 119	
ITALY	FI97A000020	7 February 1997	Yes [X]	No []
			Yes []	No []

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

¹In Non-Convention cases, a listing of all filings and current status of cases filed more than a year before the U.S. filing is required to comply with 37 CFR 1.56(a). Such a listing may be attached.

MEN. 38
JOIPTUS

-2-

APPLICATION SERIAL NO.	FILING DATE	STATUS




I hereby appoint my attorneys with full power of substitution and revocation, to prosecute this application and to transact all business in the U.S. Patent & Trademark Office connected therewith:

Edward A. Hedman, Reg. No. 22,120; Thomas M. Gibson, Reg. No. 24,638; James V. Costigan, Reg. No. 25,669; Kenneth F. Florek, Reg. No. 33,173; Alan B. Clement, Reg. No. 34,563; Martin P. Endres, Reg. No. 35,498 and Timothy X. Gibson, Reg. No. 40,618.

CORRESPONDENCE AND CALLS TO: James V. Costigan, Esq.
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New York, NY 10036-2601
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The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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1-11 1-01 Name: <u>Rosaria PIRARI</u> heir of <u>deceased inventor Raffaello</u> <u>GIORGI</u> Signature: <u>Rosaria PIRARI</u>	Date: 9 June 2000 Citizen of: ITALY	Via delle Piagge 9/b 56100 <u>PISA</u> - ITALY <u>ITX</u>
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3-11 4-11 Name: <u>Chiara GIORGI</u> heir of deceased inventor Raffaello GIORGI by <u>Rosaria PIRARI</u> as legal guardian Signature: <u>Rosaria PIRARI</u>	Date: 9 June 2000 Citizen of: ITALY	Via delle Piagge 9/b 56100 <u>PISA</u> - ITALY
5-11 6-11 Name: <u>Gabriele GIORGI</u> heir of deceased inventor Raffaello GIORGI by <u>Rosaria PIRARI</u> as legal guardian Signature: <u>Rosaria PIRARI</u>	Date: 9 June 2000 Citizen of: ITALY	Via delle Piagge 9/b 56100 <u>PISA</u> - ITALY

INVENTOR(S)	DATE	RESIDENCE AND P.O. ADDRESS
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Name: Signature:	Date: Citizen of:	
Name: Signature:	Date: Citizen of:	